A STUDY OF THE CUTANEOUS EFFECTS OF SEROTONIN*

CAPTAIN D. JOSEPH DEMIS, MC, USA, MAJOR MICHAEL J. DAVIS, MC, USA AND CAPTAIN JAMES C. LAWLER, MC, USA

Patients with metastatic carcinoid tumors may manifest a symptom complex referred to as the malignant carcinoid syndrome. These patients classically demonstrate cutaneous changes characterized by transient flushing (predominantly of the face) with areas of macular cyanosis within the flush. The central areas of the flush tend to fade, leaving erythema, blanching, and cyanosis. This phenomenon has been termed a red, white and blue response. Telangiectatic venules appear as the flushing becomes chronic. Evidence of edema formation has been noted only occasionally.

Serotonin has frequently been implicated as the agent responsible for these changes. Intraarterially (1) and intravenously (2) administered serotonin has duplicated the flush response in normal and carcinoid individuals; however, Schneckloth *et al.* (2) have reported norepinephrine and sodium nitroprusside to produce typical flushes in carcinoid patients. Intracutaneously administered serotonin has not been reported to produce these changes. Therefore, a study to evaluate the cutaneous responses to serotonin when administered intradermally and the subsequent modification of these responses by various agents was undertaken.

METHODS

Fifty healthy caucasian individuals were studied; three-fourths were young males under age 30; the remainder females and older males. No consistent differences were noted between the groups. Subjects were tested by intradermal injection of 0.1 cc. of various concentrations using disposable plastic syringes. Test sites were the volar forearm surfaces and the back. Solutions were adjusted to isotonicity with saline. Serotonin in the form of 5-hydroxytryptamine creatinine sulfate† and histamine phosphate were used with isotonic saline for controls. Concentrations are expressed in terms of the free base.

As a measure of capillary permeability, six volunteer subjects were given 25 mg. of Evans Blue dye intravenously and thirty minutes later their responses to intradermally injected materials were measured.

Cutaneous microcirculation was examined by capillary microscopy, using techniques previously described (3). Serotonin was applied by dropping it onto a site from which the keratin had been removed by stripping with cellulose tape.

RESULTS

All the subjects tested responded to both serotonin and histamine. Although specific responses to serotonin were variable, it regularly produced local erythema, a prominent persistent flare and constriction of large subcutaneous veins. Cyanosis, pain and pruritus occurred inconsistently. There was virtually no wheal formation. The molar concentrations of serotonin required to produce these effects varied over a wide range but were always greater than the concentrations of histamine required to produce comparable erythema or flare responses. In general, the molar concentrations of serotonin required were more than five times that of histamine. The particular responses to serotonin will be discussed separately.

Erythema. Serotonin in a concentration of 5 micrograms per milliliter (γ/ml) regularly produced easily visible local vasodilatation; 2 γ/ml caused erythema in three-fourths of the subjects. Concentrations as low as 0.1 γ/ml occasionally produced erythema at the injection site. This concentration approaches the concentration available in human skin (4). Local erythema appeared rapidly, becoming visible as soon as the blanching produced by intradermal injection had faded. The duration of erythema varied from 15 to 180 minutes and was approximately proportional to concentration.

The effects of various substances on serotonin-induced local vasodilatation were studied in six subjects. Following intradermal injection of the test agent, the erythema resulting from a fixed amount of serotonin and the threshold amount of serotonin required to produce erythema were

* From the Department of Dermatology, Walter Reed Army Institute of Research, Washington, D. C.

Presented at the Twentieth Annual Meeting of The Society for Investigative Dermatology, Inc., Atlantic City, N. J., June 6, 1959.

† Supplied by Sandoz Chemicals, Inc.

TABLE I

Effect of Various Agents on Serotonin-induced Local Vasodilatation

Complete Inhibition	
Norepinephrine	(1)
Epinephrine	(1)
Neosynephrine	(1)
Isoproterenol	(10)
Ergotamine tartrate	(15)
Partial Inhibition	
DCI (1-(3,4 Dichlorophenyl) 2 isopropyl-amino ethanol HCL)	(100)
Cyproheptadine	(200)
5-hydroxytryptophane	(200)
BAS (1-benzyl-2-methyl-5-methoxy tryptamine HCL)	(500)
BOL-148 (2-bromo-d-lysergic acid diethylamide tartrate)	(500)

Numbers in parentheses refer to the smallest effective concentrations, expressed as micrograms per milliliter.

TABLE II

Agents Not Affecting Serotonin-Induced Local Vasodilatation

Reserpine	(150)
Chlorpromazine	(500)
Atropine	(50)
Dibenzyline	(100)
Phentolamine	(500)
Hexamethonium	(500)
Tetraethylammonium Chloride	(10)
Tripelennamine	(25,000)
Chloroprophenpyridamine	(2,000)
Diphenhydiamine	(1,000)
Hydrocortisone	(10,000)
Desoxycorticosterone	(500)
Hexylcaine	(10,000)
Lidocaine	(10,000)
Procaine	(10,000)
Iproniazid	(500)

Numbers in parentheses refer to the greatest concentrations tested, expressed as micrograms per milliliter.

evaluated as contrasted to controls: two end points were thus utilized. The agents which inhibited erythema are listed in Table I in approximate order of potency. Complete inhibition was obtained with a variety of vasoconstrictors; these agents thus function as physiologic antagonists. Pharmacologically induced vasoconstriction is apparently a stronger cutaneous stimulus than serotonin- or histamine-induced vasodilatation for vasoconstrictors, e.g. norepinephrine 2 γ/ml, not only prevented but also diminished or blanched the erythema. White dermographism following gentle stroking did not affect the erythema but rather made it more visible by blanching the surrounding skin.

Partial inhibition was produced by a group of serotonin antagonists of varying efficacy and also by a blocker of beta adrenergic receptors (DCI). Differences between the compounds were small since these agents are weak inhibitors at best. The end point of this response is not sharp and precise evaluation is difficult, necessitating a somewhat arbitrary point of division between the weakest partial inhibitors and those with no effect. However, these agents did seem to exert a partial albeit weak effect in the six subjects tested.

Table II lists some agents which failed to affect serotonin-induced erythema. In agreement with the findings of Lorincz and Pearson (5), atropine, antihistaminics and the monamine oxidase inhibitor iproniazid failed to influence local vasodilatation.

Flare. Serotonin in a concentration of 20 γ/ml produced a flare response in all subjects; 10 γ/ml produced this response on the forearms in all but two subjects, while on the back a flare was observed with this concentration in approximately three-fourths of the individuals tested. Some subjects developed a discernible flare from concentrations as small as 1 γ/ml.

The flare response appeared within 20 to 35 seconds after injection and attained its maximum extent and intensity within five minutes. The duration was in general porportional to the amount of serotonin injected. The occasional small faint flare produced by 1 γ/ml lasted approximately 10 to 15 minutes, whereas the larger more intense flares produced by higher concentrations occasionally lasted more than three hours. In the majority of individuals, when comparable sized flares produced by histamine and serotonin were contrasted, the serotonin flare was less intense, more mottled and very indistinctly marginated. These variations are probably due to the degree of vasodilatation and presumably do not represent qualitative differences.

Many substances were tested for their ability to modify the serotonin flare. Local anesthetics and antihistamines (listed in Table II) infiltrated

into the skin in anesthetic concentrations inhibited the histamine and serotonin flares equally well, in agreement with the observations of other investigators (5, 6). In subanesthetic concentrations, only the vasoconstrictors (shown in Table I) were effective inhibitors. Norepinephrine proved most potent. In the six subjects tested none of the serotonin antagonists (Table I) and none of the agents listed in Table II modified the serotonin or histamine flares when anesthesia was not produced. These results suggest that the serotonin flare is mediated by axon reflex vasodilatation, probably of arterioles (although differential surface temperature measurements have not yet been performed). Conceivably the differences between histamine and serotonin flares may result from vasodilatation at different arteriolar levels rather than varying degrees of dilatation of the same vessels.

Cyanosis. In approximately one subject out of four, larger concentrations of serotonin produced cyanosis varying in degree from a barely detectable halo surrounding the flare to a large bluish-tinged area extending for several centimeters beyond the flare (Fig. 1). One subject routinely developed cyanosis from a single injection of 20 γ/ml; most of the susceptible individuals required from 100 to 300 γ/ml. Cyanosis was discernible within two minutes, reached a maximum in 5 to 10 minutes and usually persisted from 20 to 90 minutes, occasionally lasting 3 hours; duration was approximately proportional to concentration.

Cyanosis induced by lower concentrations of serotonin (20 to 50 γ/ml) tended to last longer than the small flare produced, whereas with higher concentrations the large, intense flare was generally of longer duration than the cyanosis. When several injections of serotonin were made in proximity to each other the cyanosis developing between injection sites was more intense than that surrounding the entire area. Cyanosis never occurred in the absence of a flare response, however this probably only reflects the larger concentrations required to produce either response.

The forearms of subjects susceptible to cyanosis gave a greater response than did the back. In some individuals, as little as 50 to 100 micrograms (0.1 ml of 0.5 to 1 mg/ml) of serotonin injected in the volar forearm led to cyanosis extending from the middle of the upper arm to the fingers. The cyanosis was interspersed with erythematous areas and as the response faded some blanching occurred. Thus a mottled red, white and blue response of the forearm was produced which, when compared to the facial cutaneous flush exhibited by two patients with functioning carcinoid tumors, closely reproduced the characteristic carcinoid appearance.

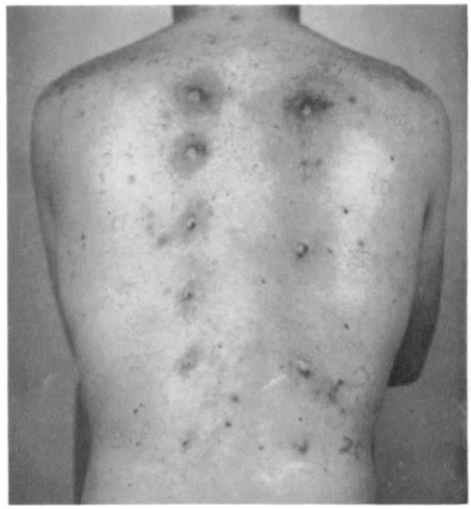

FIG. 1. The responses to intradermally injected histamine and serotonin are contrasted. On the left, histamine concentrations (from above downward) range from 33 to 2 γ/ml. An isotonic saline control is included at the bottom left. Serotonin, on the right, was injected in the following concentrations, 1000, 50, 500, 300 and 20 γ/ml, reading downward. Axon reflex flare (dark areas around the injection papules) and cyanosis induced by larger concentrations of serotonin (gray areas extending several centimeters beyond the flare) are visible. Photograph taken 6 minutes after injection.

Those dilated venules of the subpapillary plexus just visible with the naked eye, which were included in a cyanotic area became prominently congested with reduced blood, thereby assuming a telangiectatic appearance which persisted for a time after the cyanosis faded. Intracutaneous application of the agents listed in the prior tables did not prevent or reverse cyanosis. These subjects also noted numbness, paresthesias and coldness of the fingers as well as pain. In a marked response the fingers became several degrees cooler and the volume of the limb (circumference measurement) increased. Plethysmographic studies are planned.

These responses can be attributed to erythema-producing dilatation of the smaller arterioles or metarterioles coupled with venoconstriction of

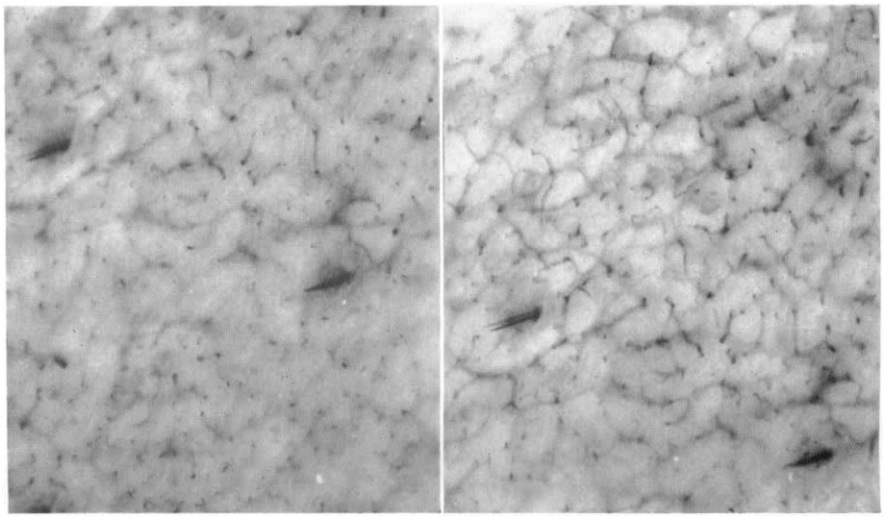

FIG. 2. Capillaries and subpapillary plexuses of forearm prior to exposure to serotonin (left). The same area (right) 10 minutes after application of serotonin (200 γ/ml). ×34.

the larger veins producing cyanosis. As a model for this hypothesis, cyanosis of an extremity was induced with a blood pressure cuff set below arterial pressure. In this situation small amounts of histamine or serotonin were able to produce erythema (though somewhat diminished) in a cyanotic skin. The slower appearance of cyanosis is attributed to the time required for serotonin to diffuse to larger veins. Diffusion losses may explain the relatively high concentrations required despite the known sensitivity of larger veins to serotonin. The decreased temperature and increased volume of the extremity can be explained by diminished venous outflow.

Venoconstriction. Locally applied serotonin produced constriction of large subcutaneous veins causing their disappearance from view, as first reported by Reid (7). Serotonin caused venoconstriction in all of the subjects tested. When placed in proximity to a vein, concentrations as small as 0.5 γ/ml applied either intradermally or by direct application to a stripped area caused short-lived vein collapse in occasional subjects. Most subjects developed venoconstriction with concentrations less than 10 γ/ml (Fig. 2). This appeared within 1 to 3 minutes and persisted as long as one hour. Two subjects required 20 γ/ml in order to induce the phenomenon. Larger amounts of serotonin resulted in venoconstriction of two to three hours or longer.

Many agents including the serotonin antagonists and the other drugs listed in the prior tables did not influence this phenomenon; none of these drugs caused venoconstriction and conversely their application prior to serotonin failed to inhibit venoconstriction. These findings are in agreement with those of Lorincz and Pearson (5); these authors were also unable to modify this phenomenon with several agents, including local anesthetics, and felt venoconstriction to be a direct action of serotonin.

Pain. Pain following serotonin injection was an inconstant finding. Only two subjects spontaneously complained of prolonged deep pain though several mentioned its presence on direct questioning. Two individuals noted tenderness of the serotonin papules to pressure without concomitant pain. Spontaneous and presumably significant pain was elicited only with the higher concentrations (500 γ/ml and above). The severity of pain which a given amount of serotonin produced was quite variable even in the same subject. Pain seemed to be most intense in subjects who developed prominent cyanosis though pain did occur without detectable cyanosis. When intense, pain was noted approximately 3 minutes after injection and often persisted more than 3 hours. Thus pain appeared shortly after and somewhat outlasted cyanosis. The deep pain may be attributed to constriction of the large veins and consequent fluid accumulation producing stretching of the tissues.

Pruritus. The majority of subjects experienced

almost immediate pruritus following injection of larger concentrations of serotonin (1 mg/ml and above). These concentrations produced whealing, often with the appearance of pseudopods, presumably by causing histamine release. Concentrations too small to induce evidence of histamine release caused pruritus no more frequently than did isotonic saline.

Wheal. In all but three subjects, serotonin in concentrations as large as 200 γ/ml did not produce detectable whealing. The serotonin injection papules became no larger and persisted no longer than saline controls. In three subjects this concentration resulted in slightly larger wheals than those produced by saline. A concentration of 300 γ/ml induced a wheal of approximately 25 per cent greater diameter than controls in one-third of subjects. A serotonin wheal twice the size of control papules was produced by a concentration of 500 γ/ml in the majority of subjects. With concentrations of 1000 γ/ml and greater, all subjects demonstrated grossly visible wheals which frequently developed pseudopods. Most of the patients noted pruritus with these concentrations and it may be assumed that serotonin had induced histamine release.

In order to facilitate study of serotonin's ability to produce whealing, volunteer subjects were given Evans Blue dye intravenously. This dye migrates with serum proteins and by staining edema fluid serves as a convenient indicator of increased capillary permeability. Serotonin was contrasted with agents known to affect the permeability of human capillaries. These included histamine and plasma permeability factor (8). In concentrations up to 100 γ/ml, serotonin failed to produce any greater color of the injection papules than did saline. In the range of 200 to

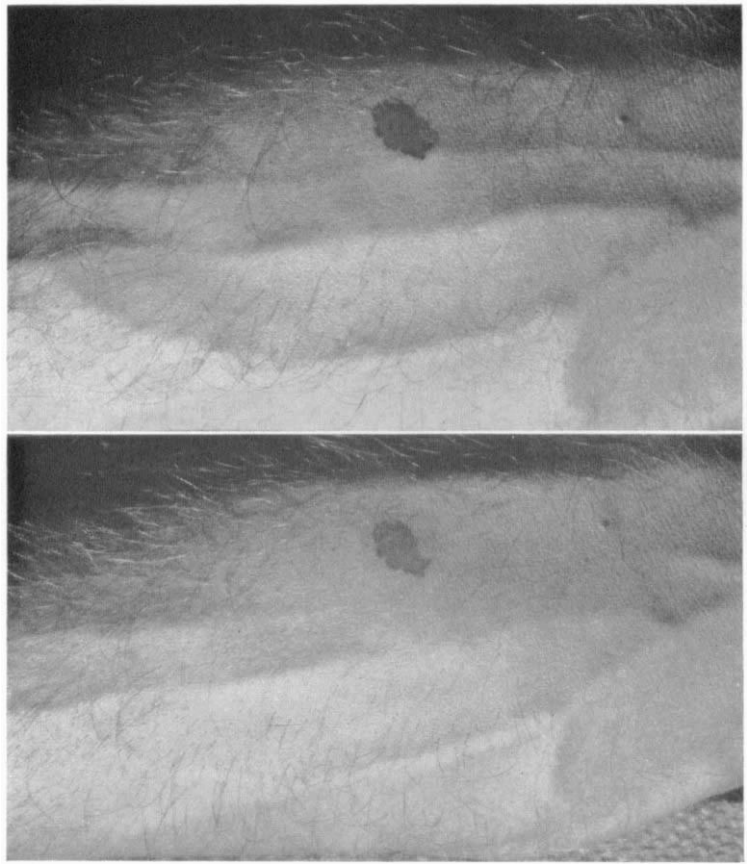

FIG. 3. Subcutaneous veins of forearm prior to serotonin (upper). Dark area is site from which keratin has been stripped away. Serotonin (100 γ/ml) was dropped onto this site. The same area (lower) 8 minutes after serotonin shows constriction of the veins.

500 γ/ml, serotonin induced a faint bluing, visible 15 minutes after injection. In order to achieve bluing as rapid or as intense as produced by 1 γ/ml of histamine, concentrations of serotonin approaching 1000 γ/ml were required. One subject responded to 750 γ/ml while one required 1500 γ/ml to achieve this degree of bluing. Histamine release, as mentioned above, is probably induced by these large concentrations. The above results are substantially in agreement with those obtained by Moller and Rorsman (9) using sodium fluorescein as an indicator of capillary transudation. These investigators found an antihistaminic compound capable of reducing the exudation, while a serotonin antagonist failed to do so, providing further evidence that serotonin effects on human capillary permeability are mediated by histamine. Thus the ability of serotonin to directly increase capillary permeability in human skin appears to be negligible.

Microcirculation. The effects of serotonin on cutaneous circulation were investigated with the aid of capillary microscopy. Serotonin was applied directly onto a site after removal of the keratin. Concentrations of serotonin up to 200 γ/ml produced a slowing in the rate of blood flow 10 to 15 minutes after application. There were no morphologic changes of the capillaries or subpapillary venules demonstrable (Fig. 3). Larger concentrations in general caused no greater response, though evidence of edema formation (haziness and pallor of the background) was occasionally noted. These changes are qualitatively similar to those observed with other vasodilators including acetylcholine and histamine.

Examination of the microcirculation in an area of serotonin-induced cyanosis revealed the rate of blood flow to be moderately slowed, and the vessels to be engorged with reduced blood. The microscopic changes were reproduced on control limbs with a tourniquet set above venous pressure, and these serotonin responses can be attributed to constriction of larger veins.

DISCUSSION

One of the pronounced responses of human skin to intracutaneously administered serotonin is constriction of large subcutaneous veins. Despite the marked response by larger veins, serotonin lacks a profound effect on smaller vessels. There is no constriction of venules of the subpapillary plexus demonstrable with capillary microscopy.

The ability to produce direct venoconstriction in man is a unique effect of serotonin and, to the best of our knowledge, is a property shared by no other available agent. This effect, first described by Reid in 1952 (7), deserves further evaluation for its significance as an important physiological function of serotonin. Venoconstriction, occurring in selected vascular areas, by influencing blood flow and pressure on the venous side of capillaries, may provide a mechanism for delicate cardiovascular control of exchange processes and metabolism.

Constriction of large subcutaneous veins occurred in every subject tested and this response is probably the basis of other observed serotonin effects, particularly pain and cyanosis. Pain has previously been reported following administration of serotonin at the base of a blister (10), where absorption may be enhanced. Cyanosis in response to intradermal serotonin has been reported by Rotbart (11) in two patients with malignant carcinoid tumors, however we were unable to demonstrate this in three patients, two of whom were experiencing typical flushes when studied. Recently, Scherbel and Harrison (12) have observed patients with rheumatoid arthritis to develop cyanosis following periarticular injection of serotonin.

Unlike venoconstriction, pain and cyanosis could not be produced in a majority of subjects studied. We do not presently understand the factors other than venoconstriction controlling cyanosis. Abnormality of serotonin metabolism does not seem to be necessarily involved since in our subjects exhibiting a marked cyanotic response, determinations of plasma serotonin and urinary 5-hydroxyindoleacetic acid were within normal limits.

Many pharmacologically active compounds including the serotonin antagonists failed to influence venoconstriction. Antiserotonin compounds do not effectively antagonize any of the responses of human skin to serotonin, nor do these compounds inhibit the effects of serotonin on the gastrointestinal tract. In this respect the receptors of the gastro-intestinal tract and human skin are similar.

The ability of serotonin to markedly increase capillary permeability was first reported by

Rawley and Benditt (13), who noted serotonin to be more effective than histamine in its ability to produce edema of the rat's paw. Sparrow and Wilhelm (14) noted marked species differences in this response; serotonin was very effective in rats but in rabbits and guinea pigs its effect was negligible. Human skin apparently responds like that of rabbits and guinea pigs for no significant increase in capillary permeability by serotonin has been demonstrated. These results make it dubious that serotonin plays any important direct role in whealing phenomena in human skin, though the possibility of an indirect adjunctive action cannot be excluded and remains to be studied. Some patients with the carcinoid syndrome have been noted to have facial and periorbital edema with severe flushing (15). This edema may be the result of venoconstriction or might be due to increased amounts of circulating histamine rather than serotonin since some of these patients are reported by Pernow and Waldenstrom (16) to excrete large amounts of histamine in the urine.

SUMMARY

Various responses to intradermally administered serotonin were studied in fifty normal subjects. All developed local erythema, a prominent protracted flare and constriction of large subcutaneous veins, although the small vessels showed no marked changes. Cyanosis developed in approximately one-fourth of the subjects while pain was only occasionally noted. Pruritus was insignificant except in association with other evidence of histamine release. Serotonin did not significantly increase capillary transudation of Evans Blue dye. Many agents were examined for their ability to inhibit responses to serotonin and while physiologic antagonisms were observed, none of the serotonin antagonists was effective in modifying the cutaneous responses. The role of venoconstriction as an important physiological function of serotonin is discussed.

REFERENCES

1. Roddie, I. C., Shepard, J. T. and Whelan, R. F.: The action of 5-Hydroxytryptamine on the blood vessels of the human hand and forearm. Brit. J. Pharmacol., **10:** 445, 1955.
2. Schneckloth, R., Page, I. H., Del Greco, F. and Corcoran, A. C.: Effects of serotonin. antagonists in normal subjects and patients with carcinoid tumors. Circulation, Off. J. of the Amer. Heart Assoc., **16:** 523–532, 1957.
3. Davis, M. J. and Lorincz, A. L.: An improved technic for capillary microscopy of the skin. J. Invest. Dermat., **28:** 283–290, 1957.
4. West, G. B. and Parratt, J. R.: 5-Hydroxytryptamine and the skin. Arch. Dermat. & Syph., **76:** 336–342, 1957.
5. Lorincz, A. L. and Pearson, R. W.: Studies on axon reflex vasodilatation and cholinergic urticaria. J. Invest. Dermat., **32:** 429–435, 1959.
6. Clendenning, W. E., DeOreo, G. A. and Stoughton, R. B.: Serotonin; Its effect in normal and atopic skin. Arch. Dermat. & Syph., **79:** 503–511, 1959.
7. Reid, G.: Circulatory effects of 5-hydroxytryptamine. J. Physiol., **118:** 435, 1952.
8. Wilhelm, D. L., Miles, A. A. and Mackay, Margaret E.: Enzyme-like globulins from serum reproducing the vascular phenomena of inflammation. Brit. J. Exper. Path., **36:** 82, 1955.
9. Moller, Halvor and Rorsman, Hans: Studies on vascular permeability factors with sodium fluorescein. III. The effect in man of intracutaneously injected 5-hydroxytryptamine. Acta dermat.-venereol., **39:** 12–16, 1959.
10. Armstrong, D., Dry, R. M. L., Keele, C. A. and Markham, J. W.: Observations on chemical excitants of cutaneous pain in man. J. Physiol., **120:** 326–351, 1953.
11. Rotbart, M.: Malignant Carcinoid Tumor: Altered Reactivity of the Skin to Serotonin in 2 patients, California Med., **88:** 112, 1958.
12. Scherbel, A. L. and Harrison, J. W.: Response to serotonin and its antagonists in patients with rheumatoid arthritis and related diseases. Angiology, **10:** 29–33, 1959.
13. Rowley, D. A. and Beneditt, E. P.: 5-hydroxytryptamine and histamine as mediators of the vascular injury produced by agents which damage mast cells in rats, J. Exper. Med., **103:** 399, 1956.
14. Sparrow, E. M. and Wilhelm, D. L.: Species differences in susceptibility to capillary permeability factors: Histamine, 5-hydroxytryptamine, and compound 48/80, J. Physiol., **137:** 51, 1957.
15. Sjoerdsma, A.: Carcinoid Syndrome, in A Textbook of Medicine, edited by Cecil, R. I. and Loeb, R. F., Philadelphia, W. B. Saunders Co., Tenth Edition, 1959.
16. Pernow, B. and Waldenstrom, J.: Determination of 5-hydroxytryptamine, 5-hydroxyindole acetic acid and histamine in thirty-three cases of carcinoid tumor (argentaffinoma). Am. J. Med., **23:** 16–25, 1957.

DISCUSSION

Dr. F. Kalz, (Montreal, Canada): I enjoyed this paper particularly, as one is always interested in hearing about subjects one has been working on oneself. There is particularly one point I should like to discuss, namely whether serotonin changes capillary permeability or not.

We have been doing similar bluing experiments and came to different results, but I must emphasize that our technic was somewhat different. We have given up Evan's Blue since this dye has several unpleasant features, chiefly we had difficulties finding volunteers who were prepared to put up with the resulting greyish blue discoloration of the skin which persists for about two weeks. We have used instead Coomassie blue, a protein stain which stains selectively plasma proteins, particularly the albumin fraction. It has a very short half life and does not stain the normal skin at all; any local serum extravasation however, shows a bright blue stain which persist for a couple of hours. If I may show two slides, with the permission of the Chairman: This series of bright blue wheals represents dilutions of serotonin, from 10 mcms/Ml up. This blue control is the histamine releaser polymixin, the saline control remains unstained. Now it is the question whether the bluing, which indicates an increase in capillary permeability is due to a direct action of serotonin upon the capillaries, or whether injection of serotonin releases histamine. We have tried to deplete an area of skin completely of histamine by means of repeated iontophoresis with the potent histamine releaser 48/80; and then to inject the test substance in this depleted area and a control area. Unfortunately, I have no data today on the behavior of serotonin in this experiment but will report the results later.

Dr. Richard B. Stoughton, (Cleveland, Ohio): Many have been interested in serotonin, including ourselves. I think one interesting point that is worth bringing up is the species difference that exists with serotonin. Rowley and Benditt (J. Exper. Med. **103:** 399, 1956) demonstrated that serotonin is a most effective agent for increasing capillary permeability in the rat. Workers today indicate from their work that serotonin does not increase capillary permeability. There have been other ancillary observations that have differed from one set of investigators to another and I wonder if perhaps this could be due to contaminants of an impure product and possibly due to differences in concentration that have been used.

Dr. Joseph Beninson (Detroit, Michigan): Many of the remarks made here today bring to mind some of the discussion which was held by some of your colleagues at the International Congress of Angiology.

Yesterday, Dr. Hadding of Northwestern discussed serotonin in the vascular bed and pointed out the same facts that Dr. Stoughton just mentioned. He noted the very precise differences of action of serotonin between species. Then, he emphasized that the effect could be different depending upon the concentrations of serotonin being used.

He described a series of experiments where giving 10 gamma of serotonin intravenously did not affect the arterioles and venules but did affect the capillaries. Since serotonin acts on the larger arteries and veins, it does not really affect the total blood flow, the total resistance, or the total blood volume that comes through a specific area of tissue.

I trust that this may throw a little more light on some of the observations that have been made here today.

Dr. D. Joseph Demis (in closing): I would like to thank all the discussants for their comments. In agreement with Dr. Beninson's remarks, we have observed little change in the capillaries with serotonin. The most pronounced cutaneous effects were exerted on the larger veins. We have not carefully studied arterial effects but have not observed constriction of the superficial arteries. However, there is variability in the responsiveness of large blood vessels to serotonin; the pulmonary vessels appear to be most responsive. Also, to reiterate what Dr. Stoughton mentioned, there is, in addition, marked species difference in responsiveness to serotonin. For example, the capillaries of rat's paw seem to be more sensitive to serotonin than are those of other species.

In reply to Dr. Kalz, our technics are different. With the quantity of Evans Blue we have employed, while the plasma retains a bluish tinge for several weeks, none of our subjects has been significantly discolored. It is difficult to understand why the use of another indicator dye should lead to such different results. The slide presented showed deeply stained wheals with concentrations of serotonin ranging upward from 10 γ/ml. The wheals all appeared to be of approximately equal intensity. Conceivably this dye might in some way induce histamine release by smaller amounts of serotonin or exert some other adjunctivant effect. We hope the explanation for these different results will become apparent with further investigation.